Evolving Bacterial Fitness with an Expanded Genetic Code

Tack, Cole, Shroff, Morrow & Ellington
Scientific Reports
2018

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Brief introduction to the topic

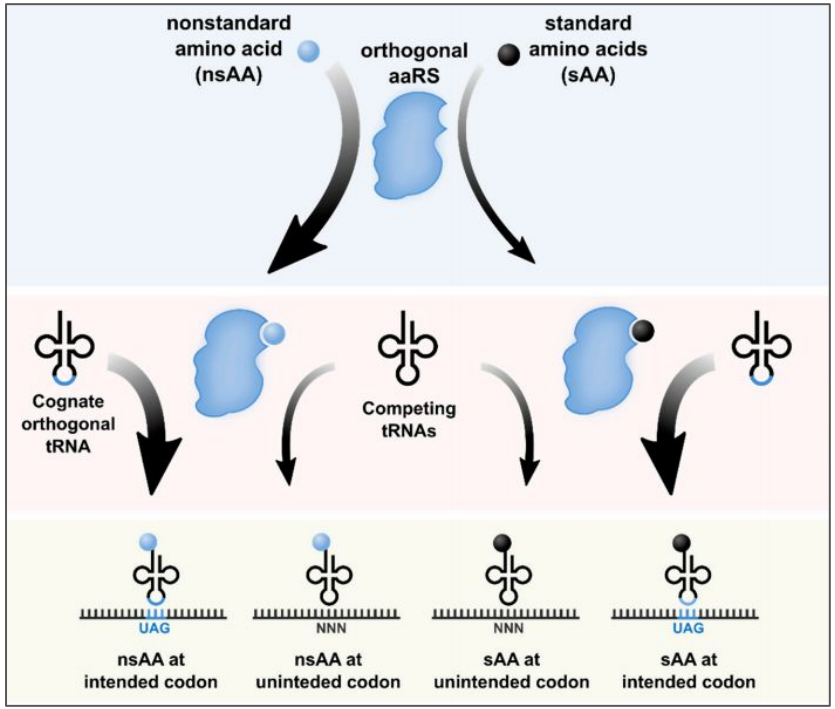
- There are 20 canonical amino acids in the standard genetic code.
- The development of the orthogonal translation systems (OTSs) allow incorporating noncanonical amino acids (ncAAs) to expand the genetic code.
- OTSs allow incorporating ncAAs by suppressing the amber stop codon (UAG).
- Cells containing an active OTS often exhibit fitness deficits.
- Some strains have found to be viable only when essential genes terminating with an amber stop codon were recoded to terminate with an alternative stop codon.

Aim of the study

- **The aim of this study was to perform directed evolution experiments with an orthogonal translation system that inserts 3-nitro-L-tyrosine (3nY) across from amber codons (UAG), to create a 21 amino acid genetic code in which the amber stop codon encodes either 3-nitro-L-tyrosine or stop.**

- The 21 amino acid code is enforced through the inclusion of an addicted, essential gene.
- After 2000 generations of directed evolution, the fitness of the strain was largely repaired.

OTS - Changing the translation machinery



From: Arranz-Gibert et al., 2019

Problem: Expand genetic code without causing cross-reactions with endogenous transcription, or translation machinery

Solution: Use enzymes from phylogenetically distant organism to favor ncAA incorporation

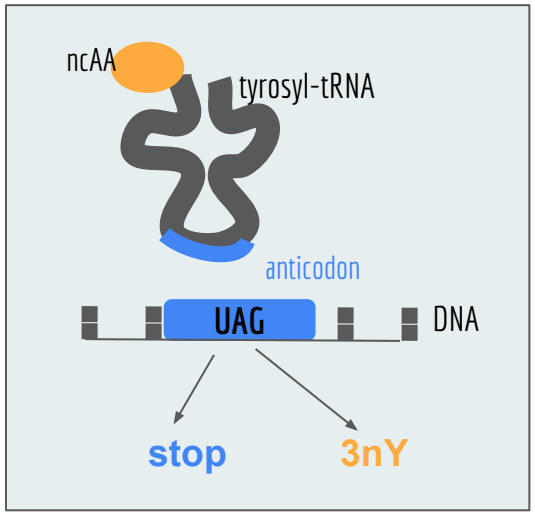
- **Engineered aaRS:tRNA pairs**
  - aaRS charges ncAA onto its cognate tRNA
  - tRNA promotes ncAA incorporation into protein

bbreviations:
aaRS = Tyrosyl-aminoacyl tRNA synthetase
ncAA/nsAA = Non-canonical/standard amino acid
sAA = Standard amino acid

Site-specific incorporation of 3nY

Heterologous components from *Methanocaldococcus jannaschii:*

- Tyrosyl-aaRS
- Suppressor tyrosyl-tRNA
  - Tyrosyl-tRNA anticodon complementary to UAG
  - Engineered to be specific for 3-iodo-L-tyrosine, compatible also with 3nY *(Sakamoto et al., 2009)*



Aim:

- Cause ambiguity in codon translation
- Stop vs. incorporation of 3nY
  - Long term: Enforce switch to 3nY alltogether

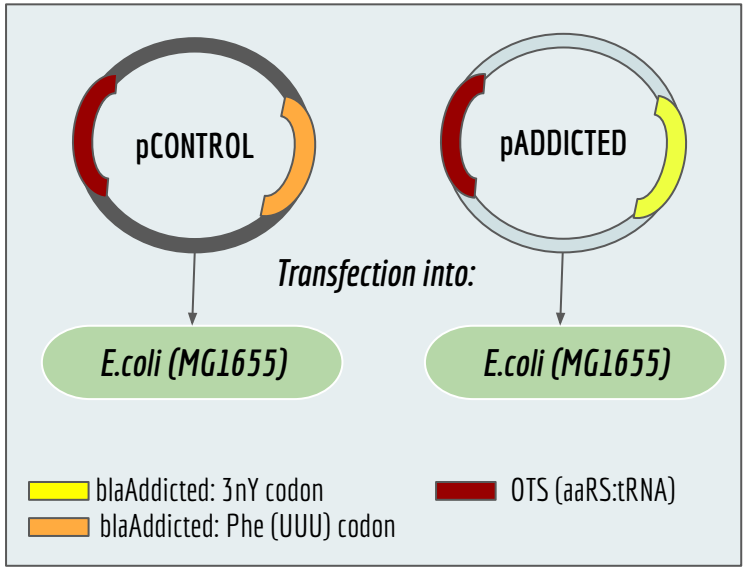
Abbreviations:
3nY = 3-nitro-L-tyrosine

OTS-addiction via a β-lactamase variant

Engineered beta-lactamase gene (*bla*):

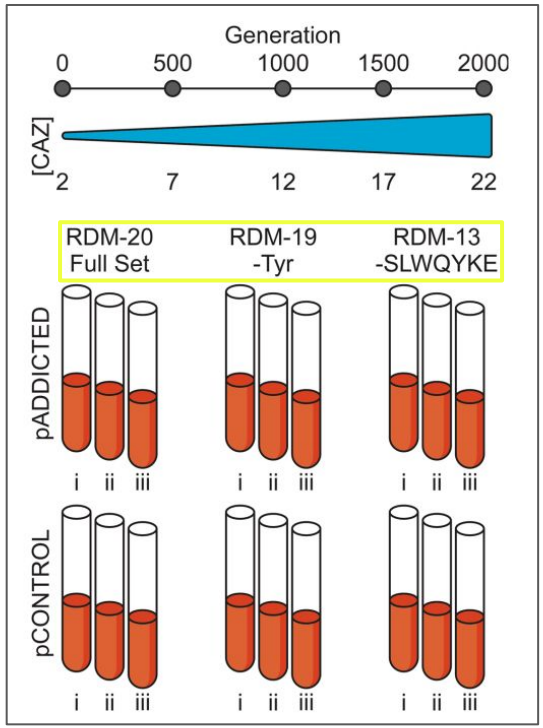
- **"*blaAddicted*" variant:**
  - Confers moderate resistance to CAZ
  - Dependency on 3nY incorporation at AA position 162

- **Control with Phenylalanine (UUU) codon:**
  - Produces functional *bla*
  - No dependency on 3nY



Abbreviations:
AA= Amino acid
CAZ = ceftazidime
Phe = Phenylalanine

Directed evolution



From: Tack et al., 2018

Transfected *E.coli* to different AA media for 2000 generations:

→*SLWQYKE:* AAs codons accessible through SNPs in UAG

→ All media supplemented with 10mM 3nY

Evolutionary pressure for 3nY retainment via:

- Progressive increase in CAZ concentration 1 µg mL−1 per 100 generations to enforce 3nY incorporation

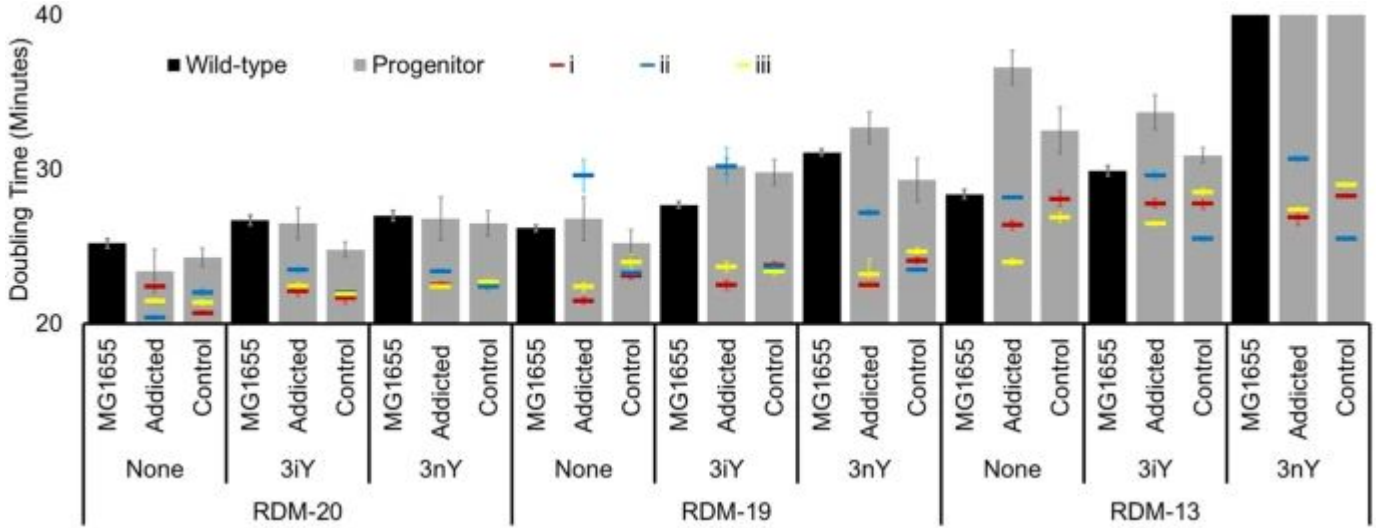
Pheno-/Genotyping via:

- Growth rates = Cellular fitness before and after evolution
- WGS = Genomic adaptation after evolution
- GFP reporter assay = OTS retention/UAG suppression efficiency before and after evolution

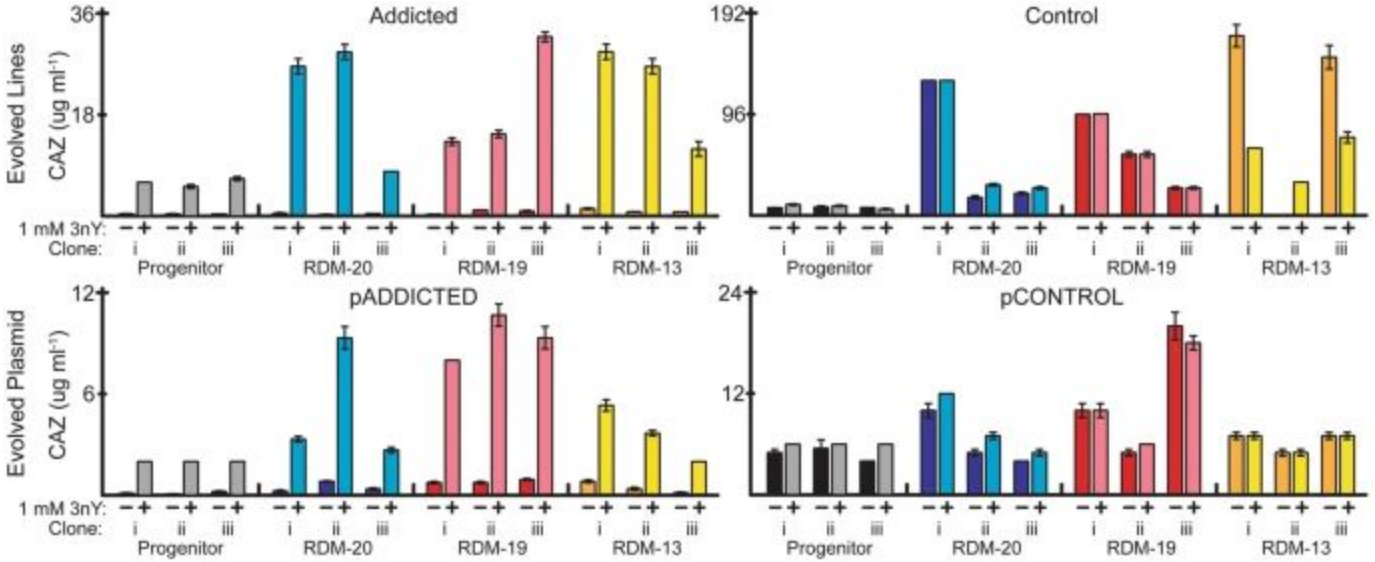
Abbreviations
SNP = Single nucleotide polymorphism
WGS = Whole genome sequencing

Growth rates as a measure of fitness

Effect of changing the media aa composition

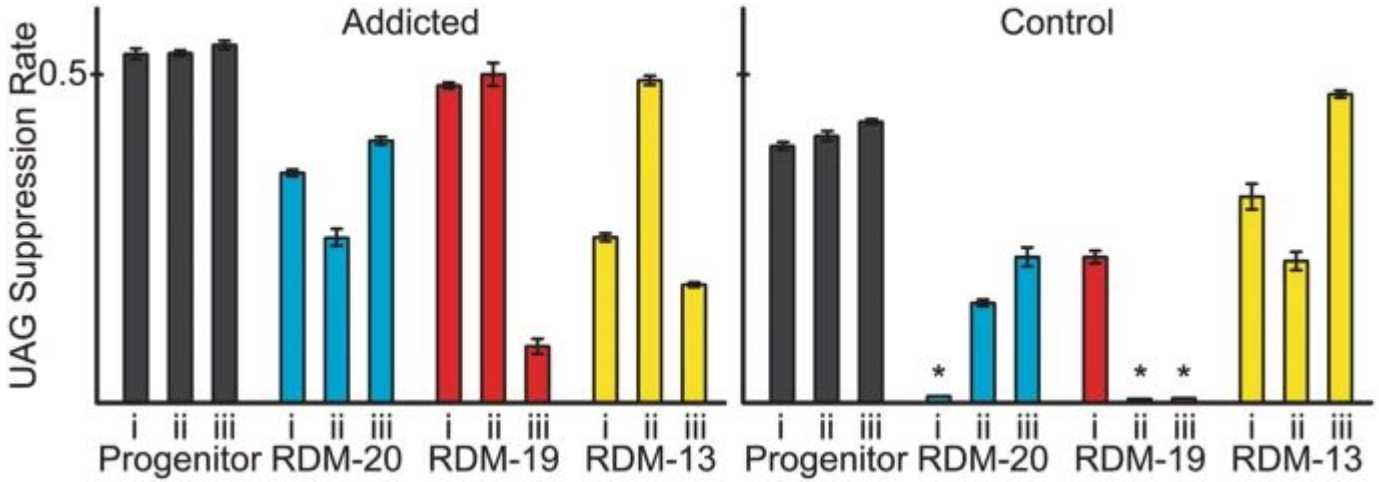


Conservation of antibiotic resistance dependency



OTS-addiction and the use of non-canonical amino acids

UAG suppression measured with GFP



Addicted lines showed conservation of OTS

Results

- Three different mixtures of amino acids environments were used (RDM-20, RDM-19, RDM-13)
- Three parameters were tested in each environment: without ncAA, with 3iY and with 3nY.
- Fitness was determined by calculating doubling times for E. Coli (MG1655) in these different mixtures.
- significant increases in doubling times was found in all three conditions(RDMs) when compared to media without ncAAs.
- the addition of 3iY to RDM-13 did not have a significant effect, with doubling times similar to growth in RDM-13 without ncAA.
- These results suggest that 3nY is mildly toxic and 3iY is less so.
- doubling times were not significantly affected by the addition of the OTS in the absence of ncAAs.

Draft

Conclusion

Created a strain with the ability to utilize a non-canonical aa

Fitness deficit repaired during only 2000 generations

Better understanding of the evolution of adoption of aa

A step toward designing unique organisms

References

Arranz-Gibert et al.: The Role of Orthogonality in Genetic Code Expansion. Life (Basel). 2019; 9(3): 58.

Tack et al.: Evolving Bacterial Fitness with an Expanded Genetic Code. Sci Rep. 2018; 8(1):3288.

Sakamoto et al.: Genetic Encoding of 3-Iodo-l-Tyrosine in Escherichia coli for Single-Wavelength Anomalous Dispersion Phasing in Protein Crystallography. Structure. 2009; 17, 335–344.